J. Fox, J. H. Burchenal and F. S. Philips, *Proc. Am. Assoc. Cancer Res.* **18**, 69 (1977).
5. J. J. Furth and S. S. Cohen, *Cancer Res.* **28**, 2061 (1968).
6. A. Čihák and J. Brouček, *Biochem. Pharmac.* **21**, 2497 (1972).
7. M-Y. Chu and G. A. Fischer, *Biochem. Pharmac.* **17**, 753 (1968).
8. M. Jurovčik, K. Raška, Jr., Z. Šormová and F. Šorm, *Colln Czech. chem. Commun. Engl. Edn* **30**, 3370 (1965).
9. M. Girard, *Methods in Enzymology*, Vol. XII, Part A, p. 581. Academic Press, New York (1967).
10. H. A. Lozeron and W. Szybalski, *Biochem, biophys. Res. Commun.* **23**, 612 (1966).
11. M. S. Zedeck, D. J. Grab and S. S. Sternberg, *Cancer Res.* **37**, 32 (1977).
12. M. G. Hamilton, *Methods in Enzymology*, Vol. XX, Part C, p. 512. Academic Press, New York (1971).
13. T. Sugimura, *Prog. nucleic Acid Res. molec. Biology* **13**, 127 (1973).
14. J. Marmur, *J. molec. Biol.* **3**, 208 (1961).
15. M. S. Zedeck and G. B. Brown, *Cancer* **40**, 2580 (1977).
16. P. Doty, *Harvey Lect.* **55**, 103 (1959–60).
17. H. Marquardt and H. Marquardt, *Cancer* **40**, 1930 (1977).
18. C. K. Chu, U. Reichman, K. A. Watanabe and J. J. Fox, *J. heterocyclic Chem.* **14**, 1119 (1977).

Biochemical Pharmacology, Vol. 28, pp. 1443–1444.

0006-2952/79/0415-1443 $02.00/0

Kinetics of phosphorylation of 5-aza-2′-deoxycytidine by deoxycytidine kinase*

(*Received* 14 *February* 1978; *accepted* 5 *October* 1978)

5-Aza-2′-deoxycytidine (5-AZA-CdR) is a potent cytotoxic agent to tumor cells *in vitro* [1] and a very effective antileukemic agent in mice [2, 3]. In general, in order for nucleoside analogs to be active inhibitors in the cell, they must first be phosphorylated. Apparently, deoxycytidine (CdR) kinase catalyzes the phosphorylation of 5-AZA-CdR since leukemic cells resistant to this analog are deficient in this enzyme [4, 5]. In this report, we have investigated the interaction of 5-AZA-CdR and its triphosphate form with CdR kinase.

[5-^{3}H]CdR and [2-^{14}C]CdR were obtained from New England Nuclear (Boston, MA). [6-^{14}C]5-AZA-CdR was synthesized by chemical methods [6] and purified by paper chromatography in *n*-butanol–H_2O (86:14). dCTP was obtained from Schwartz–Mann (Orangeburg, NY) and purified by thin-layer chromatography on DEAE-cellulose in 0.05 N HCl at 5°. 5-Aza-2′-deoxycytidine 5′-triphosphate (5-AZA-dCTP) was prepared enzymatically as described previously [7] from AZA-CdR (Chemapol, Prague). CdR kinase was purified from calf thymus as described previously [8] and had a specific activity of 100 units/mg. One unit of enzyme activity is defined as the amount of enzyme that converts 1.0 nmole of CdR to dCMP in 10 min at 37°.

The phosphorylation of CdR and AZA-CdR was assayed as described previously [8], separating the product from the substrate by adherence of the nucleotide to DEAE-cellulose discs. The reaction mixture contained in 0.1 ml: 100 mM Tris–HCl, pH 8.0, or 100 mM imidazole–HCl, pH 6.8; 5 mM ATP; 5 mM $MgCl_2$; 10 mM 2-mercaptoethanol, 0.05 μCi [^{14}C] nucleoside or 1.0 μCi [^{3}H]nucleoside and 0.3 unit CdR kinase.

In Fig. 1 is shown the Lineweaver–Burk plot of different concentrations of radioactive 5-AZA-CdR in the absence and presence of non-radioactive CdR. The apparent K_m of 5-AZA-CdR was estimated to be about 63 μM. CdR was a potent competitive inhibitor of the phosphorylation of AZA-CdR. The apparent K_i for CdR in this reaction was estimated to be 9 μM, a value which is close to its K_m value (14 μM) published previously [8]. The CdR analog, cytosine arabinoside, also has a higher K_m than the natural substrate CdR [8]. In both these cases, a structural change in the base or sugar portion of CdR produced analogs which have a lower binding affinity for the catalytic site of CdR kinase than the natural substrate.

The lower K_i of CdR as compared to the K_m of AZA-CdR is probably one of the key factors for the prevention of the antineoplastic action of this analog by CdR both *in vitro* and *in vivo* [1, 2].

Certain neoplastic cell lines resistant to the CdR analog, cytosine arabinoside, have been observed to have an increased intracellular pool of dCTP [9, 10]. Since the feedback inhibition of CdR kinase by dCTP [8] may be the biochemical mechanism of this resistance, we have investigated the effect of dCTP on the phosphorylation of AZA-CdR by CdR kinase (Table 1). In addition, we studied the effect of 5-AZA-dCTP on this reaction which could be another mechanism by which the cell modulates the intracellular pool size of 5-AZA-CdR nucleotides. At a concentration of dCTP that is within the range found in the cell [11], this nucleotide produced a significant inhibition of the phosphorylation of 5-AZA-CdR. For example, dCTP at a concentration of 10 μM produced a

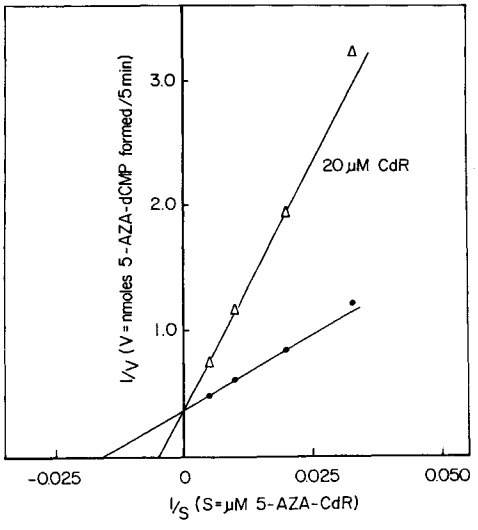

Fig. 1. Lineweaver–Burk plot of the effect of CdR on the phosphorylation of [6-^{14}C]5-AZA-CdR by CdR kinase. The standard reaction mixture with 100 mM Tris–HCl, pH 8.0, contained variable concentrations of [6-^{14}C]5-AZA-CdR. The mixture was incubated for 5 min at 37° in the presence of 0.3 unit CdR kinase and no CdR (●——●), or 20 μM CdR (△——△).

* This work was supported by Grant CH-11H from the American Cancer Society and USPHS Grant CA 23340 from the National Cancer Institute.

Table 1. Inhibition of CdR kinase by dCTP or 5-AZA-dCTP*

Substrate	Addition	Concn (μM)	Nucleotide formed (nmole)	Inhibition (%)
CdR	None		0.95	0
	dCTP	5	0.81	15
	dCTP	10	0.68	28
	dCTP	20	0.53	44
	5-AZA-dCTP	20	0.83	13
	5-AZA-dCTP	40	0.67	29
	5-AZA-dCTP	80	0.50	47
5-AZA-CdR	None		0.98	0
	dCTP	5	0.96	2
	dCTP	10	0.67	32
	dCTP	20	0.40	59
	5-AZA-dCTP	20	0.74	25
	5-AZA-dCTP	40	0.57	42
	5-AZA-dCTP	80	0.37	62

* The standard reaction mixture contained 100 mM imidazole–HCl, pH 6.8, 0.05 μCi of 20 μM [^{14}C]CdR or [6–^{14}C]5AZA-CdR, as indicated, and the indicated concentrations of dCTP or 5-AZA-dCTP. The reaction mixture was incubated for 5 min at 37° in the presence of 0.3 unit CdR kinase.

32 per cent inhibition of the phosphorylation of 5-AZA-CdR (20 μM) by CdR kinase. 5-AZA-CdR was slightly more sensitive to the inhibition produced by dCTP than the natural substrate, CdR.

5-AZA-dCTP was also an inhibitor of CdR kinase when either CdR or AZA-CdR was used as the substrate (Table 1). The inhibition of this reaction produced by 5-AZA-dCTP was less than an equimolar concentration of dCTP. Thus, this fradulent nucleotide had lower binding affinity for the enzyme than the natural nucleotide, dCTP, analogous to the condition observed for the substrates as mentioned earlier. The concentrations of 5-AZA-dCTP that inhibit CdR kinase in these experiments are probably in the range that will produce some modulation of the phosphorylation of 5-AZA-CdR at the cellular level.

Departments of Pharmacology and Pediatrics, University of Southern California, School of Medicine and Division of Hematology–Oncology, Childrens Hospital of Los Angeles, Los Angeles, CA 90027, U.S.A.

RICHARD L. MOMPARLER
DAVID DERSE

REFERENCES

1. R. L. Momparler and J. Goodman, *Cancer Res.* **37**, 1636 (1977).
2. F. Šorm and J. Veselý, *Neoplasma* **15**, 339 (1968).
3. R. L. Momparler and F. A. Gonzales, *Cancer Res.* **38**, 2673 (1978).
4. J. Veselý, A. Čihák and F. Šorm, *Colln. Czech. chem. Commun. Engl. Edn.* **34**, 901 (1969).
5. J. Veselý, A. Čihák and F. Šorm, *Cancer Res.* **30**, 2180 (1970).
6. K. K. Chan, J. A. Staroseik and W. Sadee, *J. med. Chem.* **20**, 598 (1977).
7. T. Lee and R. L. Momparler, *Analyt. Biochem.* **71**, 60 (1976).
8. R. L. Momparler and G. A. Fischer, *J. biol. Chem.* **243**, 4298 (1968).
9. R. L. Momparler, M. Y. Chu and G. A. Fischer, *Biochim. biophys. Acta* **161**, 481 (1968).
10. M. Meuth, E. Aufreiter and P. Reichard, *Eur. J. Biochem.* **71**, 39 (1976).
11. G. Bray and T. P. Brent, *Biochim. biophys. Acta* **269**, 184 (1972).

Biochemical Pharmacology, Vol. 28, pp. 1444–1446.
Pergamon Press Ltd. 1979. Printed in Great Britain.

Biochemical effects of pure isomers of hexachlorobiphenyl—Hepatic microsomal epoxide hydrase and cytosolic glutathione *S*-transferase activities in the rat*

(*Received* 25 *July* 1978; *accepted* 3 *October* 1978)

Some polychlorinated biphenyl (PCB) [1, 2] and polybrominated biphenyl isomers [3] are thought to be metabolized via arene oxides, which are usually substrates for cytosolic glutathione *S*-transferases and microsomal epoxide hydrase. PCBs have been reported to induce microsomal epoxide hydrase [4] and cytosolic glutathione *S*-transferase activities [5]. Recently, inductive properties of PCBs have been shown to be due to the combined effects of two separate classes of compounds [6], one having inducing properties similar to those of phenobarbital and the other having inducing properties similar to those of 3-methylcholanthrene. Since it is known that phenobarbital and 3-methylcholanthrene have differential effects on microsomal epoxide hydrase activity [7], the effects of 3,4,5,3′,4′,5′-HCB,† 2,4,5,2′,4′,5′-HCB and 2,3,5,2′,3′,5′-

* Part of this work was presented at the 1978 joint meeting of the American Society for Pharmacology and Experimental Therapeutics and the Society of Toxicology [*Pharmacologist* **20**, 177 (1978)].

†HCB, hepachlorobiphenyl.